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Biochemistry

SREBP-2, a second basic–helix–loop–helix–leucine zipper protein that stimulates transcription by binding to a sterol regulatory element

(cDNA cloning/cholesterol/low density lipoprotein receptor/3-hydroxy-3-methylglutaryl-coenzyme A synthase)

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ABSTRACT **We report the cDNA cloning of SREBP-2, the second member of a family of basic–helix–loop–helix–leucine zipper (bHLH-Zip) transcription factors that recognize sterol regulatory element 1 (SRE-1). SRE-1, a conditional enhancer in the promoters for the low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl-coenzyme A synthase genes, increases transcription in the absence of sterols and is inactivated when sterols accumulate. Human SREBP-2 contains 1141 amino acids and is 47% identical to human SREBP-1a, the first recognized member of this family. The resemblance includes an acidic NH_2 terminus, a highly conserved bHLH-Zip motif (71% identical), and an unusually long extension of 740 amino acids on the COOH-terminal side of the bHLH-Zip region. SREBP-2 possesses one feature lacking in SREBP-1a—namely, a glutamine-rich region (27% glutamine over 121 residues). *In vitro* SREBP-2 bound SRE-1 with the same specificity as SREBP-1a. *In vivo* it mimicked SREBP-1a in activating transcription of reporter genes containing SRE-1. As with SREBP-1a, activation by SREBP-2 occurred in the absence and presence of sterols, abolishing regulation. Cotransfection of low amounts of pSREBP-1a and pSREBP-2 into human embryonic kidney 293 cells stimulated transcription of promoters containing SRE-1 in an additive fashion. At high levels transcription reached a maximum, and the effects were no longer additive. The reason for the existence of two SREBPs and the mechanism by which they are regulated by sterols remain to be determined.**

A 10-base-pair (bp) element in the 5′ flanking region confers sterol sensitivity upon the genes encoding the low density lipoprotein (LDL) receptor and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase. This element, sterol regulatory element 1 (SRE-1), enhances transcription when cells are depleted of sterols, and loses its activity when sterols accumulate (1, 2). This feedback mechanism allows cells to satisfy their cholesterol requirement from receptor-mediated uptake of plasma lipoproteins and from endogenous synthesis, while preventing sterol overaccumulation during periods of reduced demand (3).

In recent studies a group of SRE-1-binding proteins (SREBPs) were isolated by DNA affinity chromatography from nuclear extracts of human HeLa cells (2, 4). Upon SDS/polyacrylamide gel electrophoresis, the proteins clustered in the range 59–68 kDa. The specificity of DNA binding was confirmed by the demonstration that binding correlated with transcriptional activity in a series of 16 point mutants in the SRE-1 and surrounding sequences. Each of the proteins bound to the 7 point mutants that were transcribed *in vivo*, but they failed to bind to 9 point mutants that were not transcribed (2, 4).

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Sequences of six peptides were obtained from a mixed preparation of SREBPs, and a cDNA that encoded a protein containing five of the peptides was isolated (5). The protein, designated SREBP-1, is a member of the basic–helix–loop–helix–leucine zipper (bHLH-Zip) family of transcription factors. SREBP-1 and its bHLH-Zip domain were produced by recombinant methods and shown to bind SRE-1 with perfect specificity as defined by the 16 point mutants (5). A cDNA encoding SREBP-1a activated transcription of reporter genes containing the SRE-1 when introduced into simian CV-1 cells or human embryonic kidney 293 cells by cotransfection. Surprisingly, SREBP-1a activated transcription in sterol-loaded cells as well as in sterol-depleted cells, thus abolishing sterol suppression (5).

The SREBP-1a mRNA encodes a protein of 1147 amino acids (M_r 121,666), which is much larger than the proteins purified from nuclear extracts (5). All of the sequenced peptides came from the NH_2-terminal half of the molecule, which contains the bHLH-Zip domain, suggesting that proteolysis had removed the COOH-terminal half of the purified protein. Cloning of multiple cDNAs for SREBP-1 revealed evidence of alternative splicing, which produces different sequences at the extreme NH_2 and COOH termini of the protein. The isoform that was considered most representative was designated SREBP-1a. This protein has a cluster of acidic amino acids at the NH_2 terminus, which is likely to be the transcriptional activation domain.

In the current experiments we isolated a cDNA encoding a protein that contains the single peptide that was not found in SREBP-1. This new protein, designated SREBP-2, contains 1141 amino acids, shows 47% identity to SREBP-1a, and shares the bHLH-Zip motif, the acidic NH_2-terminal domain, and the long COOH terminus.† SREBP-2 shows the same specificity for binding SRE-1 as does SREBP-1, and it also activates transcription driven by promoters that contain SRE-1. Activation occurs in the presence and absence of sterols, abolishing sterol suppression. Thus, HeLa cells have two closely related bHLH-Zip transcription factors, each of which can activate promoters containing an SRE-1 sequence.

EXPERIMENTAL PROCEDURES

Standard molecular biology techniques were used (6). DNA sequencing was performed by the dideoxy chain-termination

Abbreviations: bHLH-Zip, basic–helix–loop–helix–leucine zipper; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low density lipoprotein; SRE-1, sterol regulatory element 1; SREBP, SRE-1-binding proteins; CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus.

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†The sequence of pSREBP-2 reported in this paper has been deposited in the GenBank data base (accession no. U02031). The sequence of pSREBP-1a was reported previously (5) (accession no. U00968).

method manually or on an Applied Biosystems model 373A DNA sequencer. Blot hybridization of RNA was performed as described (5) except that three end-labeled oligonucleotides corresponding to nucleotides 598–651, 1357–1410, and 2146–2196 of human pSREBP-2 were used as probes. An antibody directed against SREBP-2 was produced by immunizing rabbits (5) with a synthetic peptide, (C)SFTQVTLPSFSPS, corresponding to amino acids 91–103 in SREBP-2 (see Fig. 1). Immunoblot analysis was performed (5) with the ECL Western blotting detection kit (Amersham). Gel mobility-shift assays were carried out with a PCR-generated ^{32}P-labeled DNA probe containing two copies of wild-type or mutant repeat 2 + 3 elements of the LDL receptor promoter (4, 5). Reporter chloramphenicol acetyltransferase (CAT) genes are described by Yokoyama *et al.* (5).

cDNA Cloning of Human SREBP-2. To obtain a cDNA probe corresponding to peptide 4 of purified SREBP (SFTQVTLPSFSPSAASPQA) (5), we synthesized four pools of degenerate oligonucleotides comprising 5′-TT(T/C)ACICA(A/G)GT(T/A/G/C)AC(T/A/G/C)(T/C)T-3′ based on the NH_2 terminus of the peptide and three pools of degenerate oligonucleotides comprising 5′-GC(T/C)TGIGG(T/A/G/C)(G/C)(A/T)(T/A/G/C)GC-3′ based on the COOH terminus of the peptide. The 12 primer pairs were used in all combinations in a two-step PCR (94°C/50°C) to amplify template DNA purified from a HeLa λgt10 cDNA library (Clontech). One primer pair, 5′-TT(T/C)ACICA(A/G)GT(C/G)AC(T/A)(T/C)T-3′ and 5′-GC(T/C)TGIGG(A/T/G/C)GA(G/C)GC-3′, yielded a 53-bp insert that was subsequently isolated from a polyacrylamide gel, reamplified, and subcloned into the pCRII vector in a TA cloning kit (Invitrogen). The translated nucleotide sequence matched peptide 4. Two partially overlapping pairs of oligonucleotides based on the 53-bp insert sequence (5′-GGTCACATTACCTTCCTTCTC-3′/5′-TTCCTTCTCTCCCTCGGC-3′, and 5′-GGAGGCCGCCGAGGGAG-3′/5′-CGAGGGAGAGAAGGAAGGTA-3′) were used together with forward and reverse primers of λgt10 (New England Biolabs) in an anchored PCR (5) performed with a "touchdown" program (7) to amplify the adjacent sequences of the insert from the HeLa λgt10 cDNA library. Fragments of 168 and 348 bp were amplified from the 5′ and 3′ ends of the insert, respectively. The two amplified fragments were ^{32}P-labeled by

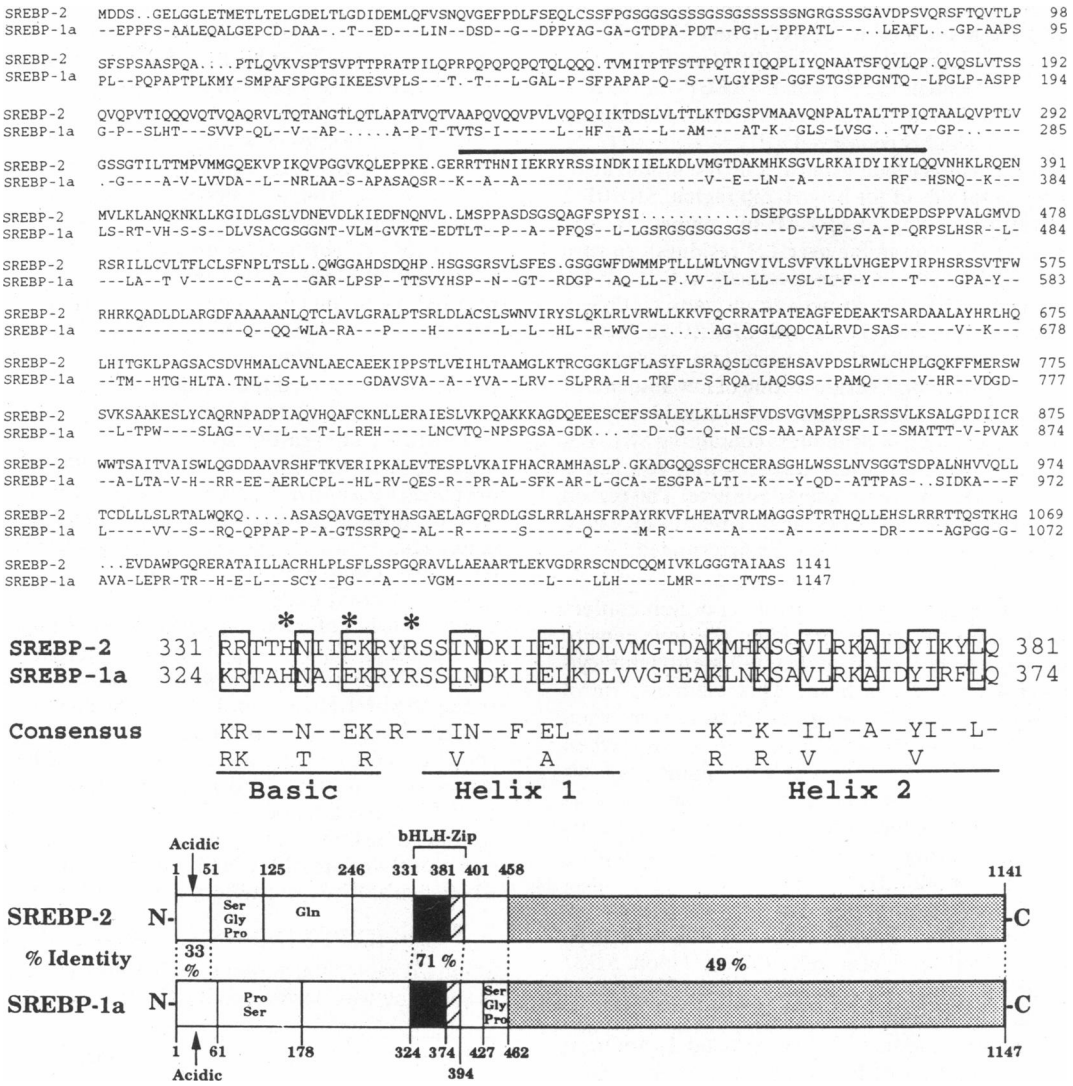

FIG. 1. Comparison of the amino acid sequences and domain structures of human SREBP-2 and SREBP-1a. (*Top*) Dashes denote residues in SREBP-1a that are identical to SREBP-2. The overline denotes the bHLH-Zip region. (*Middle*) Comparison of the bHLH region of SREBP-2 and -1a with the consensus sequence of other bHLH proteins (10, 11). Asterisks denote amino acids in Max that contact specific nucleotides in its recognition sequence as determined by x-ray crystallography (10). (*Bottom*) Domain structure of SREBP-2 and -1a with numbers corresponding to the amino acid residues (5). The bHLH region is denoted by the black box, the leucine zipper region by the hatched box, and the COOH-terminal domain by the stippled box. Regions rich in particular amino acids are indicated. The percent identities in the three most homologous regions are indicated.

the random primer method and used to screen a HeLa λEXlox cDNA library (5). Eleven positive clones were obtained from 4.2×10^5 plaques. One 4.2-kb clone, pXH-4, was sequenced on both strands. To screen for longer cDNAs, we generated a PCR fragment corresponding to the 5'-most 258 bp of pXH-4. This 258-bp fragment was ^{32}P-labeled by the random primer method and used to screen 5.2×10^5 plaques from the HeLa λEXlox cDNA library. Twenty positive clones were obtained. The longest clone, pXH-11, was ≈5.2 kb long. It did not extend past the 5' end of pXH-4. Rather, it extended further in the 3' direction. The 3' untranslated region of pXH-11 (1.1 kb) was sequenced on both strands.

Transfection and CAT Assays. An expression vector for SREBP-2, designated pSREBP-2, was constructed by cloning the 4.2-kb *Sal* I insert from pXH-4 into the *Sal* I site of pCMV7 (8). Monolayers of human embryonic kidney 293 cells were cotransfected with 0–0.6 μg of pSREBP-1a or pSREBP-2, 0.3 μg of pVA, and 1 μg of the indicated reporter CAT gene as described (5). The total amount of cDNA was adjusted to 3 μg by addition of pCMV7 (vector control) and salmon sperm DNA. After 2 days the cells were harvested and CAT activity was measured by the xylene extraction method (2). Protein content was measured by the method of Bradford (9).

Production of Recombinant bHLH-Zip Domain of SREBP-2. The nucleotide sequence corresponding to amino acids 48–403 of SREBP-2 was amplified by PCR, subcloned between the *Bam*HI and *Hin*dIII sites of a pQE-30 vector (containing six consecutive histidines after the initiator methionine) (Qiagen, Chatsworth, CA), and expressed in *Escherichia coli* as described (5). The resulting fusion protein was purified by Ni^{2+}-Sepharose affinity chromatography (5).

RESULTS

We previously reported the sequence of six peptides obtained from a mixture of SREBPs isolated from HeLa cells (5). DNA sequences encoding five of the six peptides were found in SREBP-1 (5). Using a strategy based on sequential PCR applied to a HeLa cell cDNA library, we isolated a cDNA encoding the single peptide (peptide 4) that was missing from SREBP-1 (see *Experimental Procedures*). The longest of the initial clones, pXH-4, was 4.2 kb long (nucleotides 1–4249) and encoded a protein of 1141 amino acids. This cDNA had a putative 5' untranslated region of 117 bp; it lacked a terminator codon upstream of the first in-frame methionine. To make certain that the first methionine was indeed the initiator, we rescreened the library by using a probe corresponding to the 5' end of pXH-4. The longest clone isolated (pXH-11) was 5.2 kb long and did not encode additional 5' sequence (nucleotides 88–5197). Rather, it extended further in the 3' direction, terminating in a poly(A) tract.

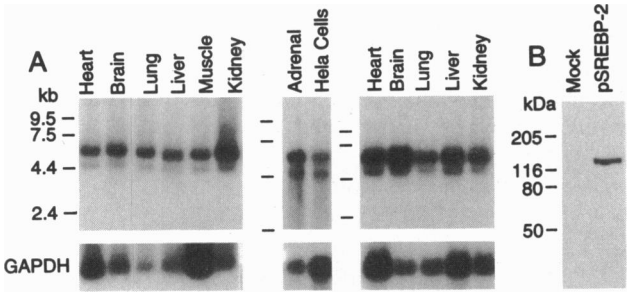

FIG. 2. Human SREBP-2. (*A*) Tissue distribution of mRNA. ^{32}P-labeled oligonucleotide probes were hybridized to poly(A)$^+$ RNA [2 μg per lane (*Left* and *Right*) or 2.5 μg per lane (*Middle*)] from the indicated human adult (*Left* and *Middle*) or fetal (*Right*) tissue. The filters were exposed to Kodak XAR-5 film with an intensifying screen at −70°C for 16 hr. The same filters were subsequently hybridized with a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe and exposed for 2 hr. (*B*) Immunoblot analysis in transfected 293 cells. A cDNA encoding human SREBP-2 driven by the CMV promoter was introduced into human embryonic kidney 293 cells by transfection. After 40 hr in medium containing 10% fetal bovine serum, cell extracts were subjected to SDS/7.5% polyacrylamide gel electrophoresis, transferred to nitrocellulose filters, and incubated with rabbit anti-SREBP-2 IgG (5 μg/ml), followed by anti-rabbit IgG conjugated to horseradish peroxidase (ECL Western kit, Amersham). The filter was exposed to Kodak XAR-5 film for 5 sec at room temperature. The positions of prestained molecular size markers are indicated. Left lane, 10 μg of protein from cells transfected with 3 μg of pCMV7 (control); right lane, 5 μg of protein from cells transfected with 3 μg of pSREBP-2.

An expressible cDNA was constructed from pXH-4. The sequence of this cDNA, designated pSREBP-2, includes a 5' untranslated region of 117 bp, an open reading frame of 3423 bp, and a 3' untranslated region of 706 bp. We believe that pXH-11 represents a full-length cDNA because its length corresponds to the longest mRNA detected on Northern blots (see below) and because the initiator methionine occurs in a position corresponding to the initiator methionine in SREBP-1a (see Fig. 1). SREBP-2 contains 1141 amino acids. Residues 91–109 correspond exactly to the sequence of peptide 4 derived from the purified SREBP preparation.

Fig. 1 compares the amino acid sequences and domain maps of SREBP-1a and SREBP-2. The two proteins share 47% of their amino acids in common, and the identities extend throughout the proteins. In the bHLH-Zip region the proteins are 71% identical, and SREBP-2 shares all of the important amino acids that form the bHLH-Zip consensus. SREBP-2 also shares the histidine, glutamic, and arginine residues (asterisks in Fig. 1) that are found in SREBP-1 (5) and that have been implicated in DNA recognition by Max, another bHLH-ZIP protein (10). Both proteins have a pair of negative residues immediately following the initiator methionine. Although the NH_2-terminal ends of the two proteins are only 33% identical, they share an extremely acidic character. The NH_2-terminal 51 amino acids of SREBP-2 include 14 negative residues (27%). The first basic amino acid (an arginine) does not occur until position 77. This is similar to the situation with SREBP-1a, in which the first basic residue occurs at position 108 (5). The acidic region of SREBP-2 is followed by a region in which serine, proline, and glycine comprise 60% of the residues. The corresponding region of SREBP-1a is also rich in proline and serine, but not in glycine. SREBP-2 contains one feature not present in SREBP-1, a glutamine-rich (27%) region at residues 125–246. The COOH-terminal halves of the two proteins share long stretches of identity (49% identical over 684 residues).

The mRNA for SREBP-2, like that for SREBP-1 (5), is expressed in a wide variety of human tissues as revealed by Northern blots (Fig. 2*A*). In most tissues the predominant mRNA is 5.2 kb long, corresponding to pXH-11. A less abundant mRNA of ≈4.2 kb is also seen. We believe that this corresponds to pXH-4 and represents the use of an upstream polyadenylylation signal. The mRNA is also expressed widely in human fetal tissues, as shown in the five lanes at the right of Fig. 2*A*.

The sequence of pXH-4 was inserted into an expression plasmid under control of the cytomegalovirus (CMV) promoter and introduced into human 293 cells by transfection. Cell extracts were subjected to SDS/polyacrylamide gel electrophoresis and immunoblotting with an anti-peptide antibody directed against SREBP-2. The cells produced a protein of ≈120 kDa (Fig. 2*B*), consistent with the predicted molecular weight of SREBP-2, which is 123,665.

In the LDL receptor promoter the SRE-1 is contained within a 16-bp sequence designated repeat 2 that is immediately followed by a related 16-bp sequence designated repeat 3 (1–3). Repeat 3 does not bind SREBP but contains a binding

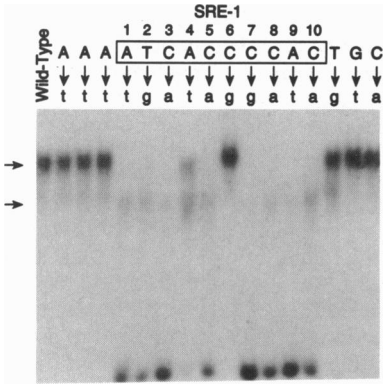

FIG. 3. DNA-binding activity of recombinant bHLH-Zip domain of SREBP-2. Aliquots (2 μg) of the purified recombinant bHLH-ZIP domain of SREBP-2 were incubated in a gel mobility-shift assay (*Experimental Procedures* and ref. 5) for 20 min at room temperature with the indicated wild-type or mutant ^{32}P-labeled, PCR-derived DNA probe (94 bp). Each probe (4×10^4 cpm per reaction mixture) contained two copies of repeats 2 and 3 with the indicated point mutation in each copy of repeat 2 (4, 5). After electrophoresis, the gel was exposed to Kodak XAR film for 1 hr at −80°C with an intensifying screen. The SRE-1 sequence within repeat 2 is boxed. Lower and upper arrows denote protein bound to one or two copies of SRE-1, respectively (4).

site for another transcription factor, Sp1 (2, 12). High-level transcription requires repeats 2 and 3. To test the binding activity of SREBP-2, we inserted a portion of the cDNA encoding the bHLH-Zip region into an *E. coli* expression vector. The protein was produced with an NH_2-terminal extension of six histidine residues that permitted isolation on a Ni^{2+}-agarose column. The purified protein gave a retarded band when incubated with a ^{32}P-labeled oligonucleotide containing two tandem copies of repeats 2 and 3 (Fig. 3). Binding was markedly reduced or abolished when any one of the nucleotides in the SRE-1 was subjected to transversion mutation with the exception of the C at position 6, which is the only one of the SRE-1 mutants that is actively transcribed *in vivo* (2). A small amount of binding was observed when the A at position 4 was mutated to a T. This sequence gives rise to low but detectable transcription *in vivo* (2). Mutations in the repeat 2 sequences flanking SRE-1 had no effect on SREBP-2 binding.

To test the activity of SREBP-2 on gene transcription, we used a mammalian expression vector that produces SREBP-2 driven by the CMV promoter (see Fig. 2*B*) and a similar vector that produces SREBP-1a (5). These constructs, or a control pCMV vector, were introduced into human 293 cells together with plasmids containing a CAT transcription reporter gene driven by promoters that contain SRE-1. When transfected together with pCMV, plasmid K, which contains two tandem copies of repeats 2 and 3 (2), was transcribed in the absence of sterols and repressed by sterols (Fig. 4*A*). Cotransfection with either pSREBP-2 or pSREBP-1a markedly enhanced transcription in the absence and presence of sterols, abolishing sterol suppression. This stimulation was not observed with plasmid X, which contains a point mutation that inactivates the SRE-1 (Fig. 4*B*). The promoters for HMG-CoA synthase (Fig. 4*C*) and the LDL receptor (Fig. 4*D*) were also stimulated by pSREBP-2 to a similar extent as pSREBP-1a. There was no significant stimulation of the promoter for HMG-CoA reductase, which does not contain SRE-1 (Fig. 4*E*).

The data of Fig. 4 suggest that SREBP-2 and -1a act similarly in stimulating transcription driven by promoters that contain SRE-1. To determine whether the effects were additive, we performed a titration experiment (Fig. 5). Human 293 cells were cotransfected with Plasmid K plus increasing concentrations of pSREBP-2 alone or together with a fixed amount (0.02 μg) of pSREBP-1a. As a blank value for the CAT assays, we subtracted the activity observed in the absence of either SREBP, and therefore the data represent the SREBP-stimulated transcription activity. The dashed line in Fig. 5 indicates the CAT activity to be expected if the actions of SREBP-1a and -2 were additive. In the absence of sterols, increasing amounts pSREBP-2 gave a nearly linear increase of CAT activity up to 100 nmol·min^{-1}·mg^{-1} (Fig. 5*A*, ●). pSREBP-1a by itself increased CAT activity by 40 nmol·min^{-1}·mg^{-1}. In the presence of SREBP-1a, the further addition of pSREBP-2 in amounts up to 0.2 μg gave an additive effect, reaching a plateau at 80 nmol·min^{-1}·mg^{-1} (▲). Increasing pSREBP-2 above this level did not produce a further increase in CAT activity. Similar results were obtained in the presence of sterols (Fig. 5*B*).

DISCUSSION

The current data reveal the primary structure and activity profile of SREBP-2, the second member of a family of

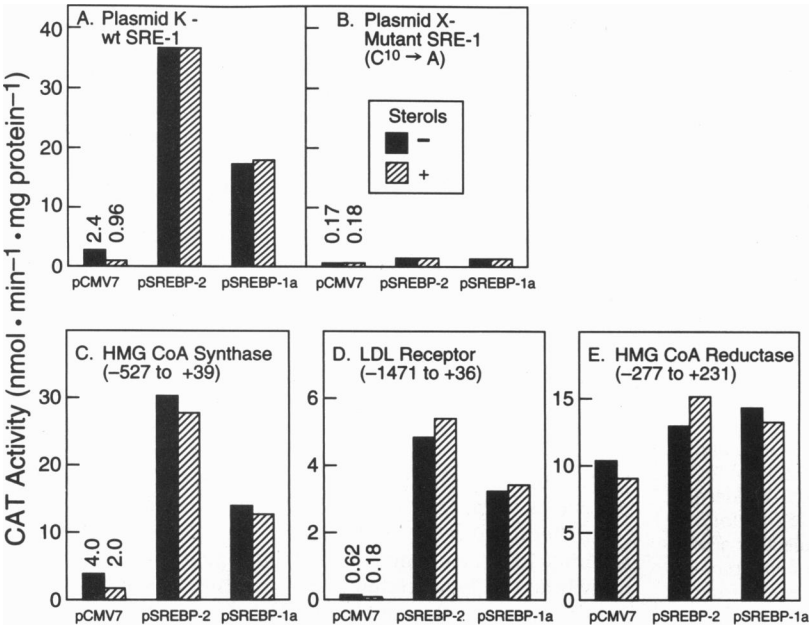

FIG. 4. Effect of SREBP-2 and -1a on transcription as shown by CAT reporter assays in transfected 293 cells. Cells were cotransfected with 0.3 μg of the indicated plasmid and 1 μg of a reporter CAT gene driven by one of the following promoter elements: synthetic sequence containing two tandem copies of repeats 2 and 3 with wild-type SRE-1 (plasmid K) (*A*); mutant sequence containing two tandem copies of repeats 2 and 3 with point mutation in each SRE-1 (plasmid X) (*B*); native HMG-CoA synthase, nucleotides −527 to +39 (plasmid J) (*C*); native LDL receptor, nucleotides −1471 to +36 (p1471) (*D*); or native HMG-CoA reductase, nucleotides −277 to +231 (pRedCAT-1) (*E*). After incubation for 40 hr in the absence (filled bars) or presence (hatched bars) of cholesterol (10 μg/ml) plus 25-hydroxycholesterol (1 μg/ml), duplicate dishes of cells were harvested for measurement of CAT activity. The absolute values for the control pCMV7-transfected cells in *A–D* are shown above the bars. All data are from the same transfection experiment.

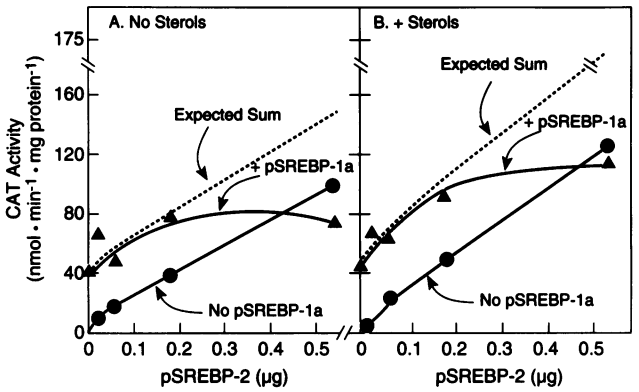

FIG. 5. Additivity of pSREBP-2 and -1a on transcription of LDL receptor promoter–CAT reporter gene in transfected 293 cells. Cells were cotransfected with the indicated amount of pSREBP-2 in the absence (●) or presence (▲) of 0.02 μg of pSREBP-1a and 1 μg of a reporter CAT gene containing two tandem copies of repeats 2 and 3 (plasmid K). After incubation for 40 hr in the absence (*A*) or presence (*B*) of cholesterol (10 μg/ml) plus 25-hydroxycholesterol (1 μg/ml), duplicate dishes of cells were harvested for measurement of CAT activity. Assays were carried out under conditions (0.25 μg of extract protein; 12 min at 37°C) in which <20% of the [^{14}C]chloramphenicol had been converted to butyrylated products. A blank value from cells cotransfected with the reporter CAT gene and pCMV (vector control) was subtracted from each value in *A* (9.7 nmol·min^{-1}·mg^{-1}) and *B* (2.6 nmol·min^{-1}·mg^{-1}). Dotted line denotes the expected sum of CAT activity generated by pSREBP-2 plus pSREBP-1a.

SREBPs. The sequence and biologic activities of this protein resemble those of SREBP-1a, the previously described member of this family (5). The reason for the existence of two SREBPs is not yet clear. Both proteins are likely to be expressed in the same cells, as evidenced by the observations that mRNAs for SREBP-1a and 2 are present in the same tissues and that both proteins were purified and their cDNAs cloned from HeLa cells.

bHLH proteins, with or without leucine zippers, usually act by forming homo- or heterodimers (10, 13). We have preliminary evidence that SREBP-1a can form homodimers and higher-order homomultimers (unpublished data). To date, we have not examined potential interactions between SREBP-1a and -2. Each is able to bind DNA independently, as revealed by gel retardation assays using recombinant proteins prepared in *E. coli* or translated in reticulocyte lysates (ref. 5; unpublished data). Moreover, each independently stimulates transcription from SRE-1-containing promoters in transfected cells. The latter conclusion must be tempered because we cannot rule out the formal possibility that the active species is a heterodimer between the protein produced by the transfected cDNA and an endogenous partner.

When transfected together at low concentrations, pSREBP-1a and -2 produce additive effects on transcription as indicated by CAT assays (Fig. 5). At higher concentrations a plateau is reached, and additivity is no longer apparent. The simplest explanation is that each protein is capable of binding to SRE-1 and activating transcription independently, and that the SRE-1 eventually becomes saturated with one or the other SREBP, limiting any further increase. Whether transfected separately or together, SREBP-1a and SREBP-2 abolish sterol suppression.

The one striking difference between SREBP-2 and -1a is the glutamine-rich region in the former. Glutamine-rich regions are reported to activate transcription, presumably by interacting with coactivator proteins (14). Both SREBP-1a and SREBP-2 have acidic NH_2 termini, which are likely to be transcriptional activating domains. It is possible that the additional glutamine-rich domain in SREBP-2 allows it to interact with an additional coactivator and thereby to produce effects that are different from those of SREBP-1a. Although these differences were not apparent in the current study, our experiments were conducted under highly artificial conditions.

The mechanism by which sterols reduce the activities of SREBP-1 and -2 remains obscure. We hypothesize that this control is abolished in transfected cells because some component of the regulatory machinery is overwhelmed by the large amount of SREBP that is produced.

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1. Smith, J. R., Osborne, T. F., Goldstein, J. L. & Brown, M. S. (1990) *J. Biol. Chem.* **265**, 2306–2310.
2. Briggs, M. R., Yokoyama, C., Wang, X., Brown, M. S. & Goldstein, J. L. (1993) *J. Biol. Chem.* **268**, 14490–14496.
3. Goldstein, J. L. & Brown, M. S. (1990) *Nature (London)* **343**, 425–430.
4. Wang, X., Briggs, M. R., Hua, X., Yokoyama, C., Goldstein, J. L. & Brown, M. S. (1993) *J. Biol. Chem.* **268**, 14497–14504.
5. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein, J. L. & Brown, M. S. (1993) *Cell* **75**, 187–197.
6. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
7. Don, R. H., Cox, P. T., Wainwright, B. J., Baker, K. & Mattick, J. S. (1991) *Nucleic Acids Res.* **19**, 4008.
8. Andersson, S., Davis, D. L., Dahlback, H., Jornvall, H. & Russell, D. W. (1989) *J. Biol. Chem.* **264**, 8222–8229.
9. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
10. Ferré-D'Amaré, A. R., Prendergast, G. C., Ziff, E. B. & Burley, S. K. (1993) *Nature (London)* **363**, 38–45.
11. Prendergast, G. C., Lawe, D. & Ziff, E. B. (1991) *Cell* **65**, 395–407.
12. Dawson, P. A., Hofmann, S. L., van der Westhuyzen, D. R., Brown, M. S. & Goldstein, J. L. (1988) *J. Biol. Chem.* **263**, 3372–3379.
13. Murre, C. & Baltimore, D. (1992) in *Transcriptional Regulation*, eds. McKnight, S. L. & Yamamoto, K. R. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 861–879.
14. Pugh, B. F. & Tjian, R. (1990) *Cell* **61**, 1187–1197.